Investigation of Urolithiasis Occurring In Hatchery-Reared Trout in Utah

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INVESTIGATION OF UROLITHIASIS OCCURRING IN HATCHERY-REARED TROUT IN UTAH

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

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Fishery Biology

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Ernest H. Dean, Jr.

ABSTRACT

Investigation of Urolithiasis Occurring In

Hatchery-Reared Trout in Utah

by

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Utah State University, 1971

Major Professor: Dr. William F. Sigler
Department: Fishery Biology

The urolithiasis condition investigated occurred at a "hard water" hatchery in Utah after the trout were changed from a meat diet to a commercial pelleted fish feed as a sole sustaining diet in 1961. Numerous diet modifications proved unsuccessful in preventing urolithiasis at the hatchery. It was eventually determined that different commercial diets resulted in varied percentages of trout developing urolithiasis. The urolithic deposits found in the kidney tubules were amorphous and composed of calcium phosphate (apatite) and an organic matrix. Bacterial contamination of the kidneys was not an important factor in the formation of urolithiasis. Rainbow trout containing urolithic deposits showed gradual deposit regression when transferred into a "softer" water supply.

Pelleted experimental diets containing sodium bicarbonate and/or sodium fluoride were fed to two strains of rainbow trout reared in a "softer" water supply normally causing no urolithiasis problems. The diets with the sodium bicarbonate added resulted in a significant occurrence of urolithiasis when fed to trout for 30 days. The addition of sodium fluoride to the diet was not significant in producing urolithiasis in trout. One experimental diet (low sodium bicarbonate-sodium fluoride) and one strain of fish (Soap Lake) appeared to influence the number of trout developing urolithiasis although the increase was not significant. All levels of statistical significance were tested at the 5 percent level.

(56 pages)

INTRODUCTION

Urolithiasis was first detected as a problem in Utah, in rainbow trout (*Salmo gairdneri*), at the State Midway Fish Culture Station during 1961. Trout reared at this hatchery were observed with urolithic deposits in their kidneys (Figures 1 and 2). Mineral deposits were also found in the pseudobranchia of these fish (Figure 3). The trout showed no external symptoms or signs of the urolithic condition except the possible presence of deposits in the pseudobranchia. Some groups of adult fish (over one year old) reared at the affected hatchery showed an incidence as high as 90 percent with renal damage from urolithiasis.

Because of the internal appearance of the kidneys, many trout with urolithiasis were not utilized when caught by fishermen, so affected fish represented a loss to the Utah Division of Wildlife Resources. This condition was also considered as possibly contributing to the disease problems at the Midway Hatchery.

Rainbow trout comprise the vast majority of the production of the hatchery, thereby being the primary species affected. However, brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Salmo clarki*) reared at this hatchery also developed mineral deposits. Other species have been reared at this hatchery, but have always been stocked throughout the waters of the state before reaching a size where they might develop similar problems.

The internal deposits were typical in all affected fish, with the first grossly visible signs appearing as white spots, thin white lines,

Figure 1. Initial gross urolithic deposits in the posterior kidney of an 8 inch rainbow trout reared at Midway Hatchery.

Figure 2. Advanced urolithiasis found in an 8 inch rainbow trout reared at Midway Hatchery.

Figure 3. Lithic deposits located in the pseudobranch of a rainbow trout reared at Midway Hatchery.

or worm-like patterns in the posterior portion of the kidneys. The posterior portion of the kidney always showed the greatest accumulation of urolithic material, but the deposits often extended anteriorly along the entire kidney. Excessive deposits were frequently observed within the kidneys, often causing the kidneys to become swollen many times their normal size.

When examined, the typical affected kidneys were found to contain a light-colored cheesy mass consisting of amorphous mineral deposits located in swollen or ruptured kidney tubules (Figures 4 and 5). Later stages showed extensive fibrous tissue accumulation.

The urolithiasis problem at this hatchery first became noticeable after changing to dry fish diets as a complete feed. These same diets were also used at the remaining ten state fish hatcheries. Observation and analysis of fish at the other ten hatcheries indicated that the problem was almost completely limited to the one hatchery. Analysis showed the water at the affected hatchery was the hardest water supply (had more soluble salts) of all state hatcheries (Table 1). The problem was initially considered to be a result of water chemistry or quality interacting with some portion or portions of dry trout diets.

The problem persisted, and the present study, started in 1966, is a continuation of research designed to determine the causative factors and was initiated by the Division of Wildlife Resources, Utah State Department of Natural Resources.

The research consisted of the following four main investigations into the problem of urolithiasis in rainbow trout:

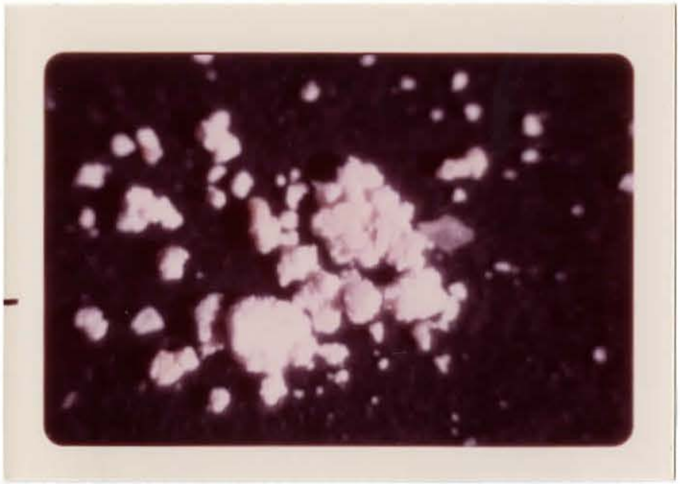

Figure 4. Urolithic deposits removed from kidneys of a rainbow trout reared at Midway Hatchery.

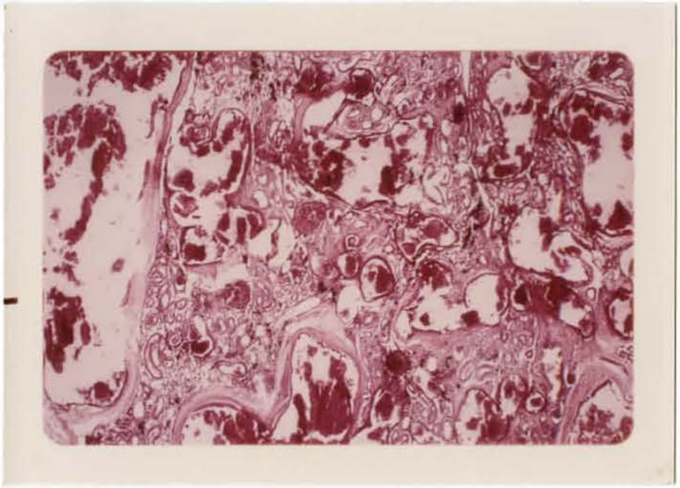

Figure 5. Histological section of a rainbow trout kidney containing advanced urolithiasis. The extensive deposition of material has replaced the normal renal tissue. H & E stain.

Table 1. Dissolved constituents and properties of water supplies used for fish culture in Utah, based on samples collected during the period of March 1961 to June 1963.

Chemical constituent or property	Range in hatchery waters where urolithiasis is no problem	Range in Midway Hatchery waters where urolithiasis is a problem
Silica (SiO_2), ppm	6.0 - 44	12 - 21
Iron, ppm	.00 - .14	.00 - .03
Manganese, ppm	.00 - .13	-
Copper, ppm	.71 - 1.4	3.4
Molybdenum, ppm	.19 - 1.3	.78
Calcium, ppm	26 - 128	146 - 193
Magnesium, ppm	4.6 - 37	24 - 41
Sodium, ppm	3.3 - 43	32 - 55
Potassium, ppm	.7 - 3.5	8.2 - 13
Ammonium (NH_4), ppm	.00 - .14	.00 - .04
Bicarbonate (HCO_3), ppm	114 - 289	331 - 460
Sulfate (SO_4), ppm	3.2 - 231	223 - 332
Chloride (Cl), ppm	1.0 - 53	30 - 51
Fluoride (F), ppm	.1 - .5	.8 - 1.1
Nitrite (NO_2), ppm	.00 - 19	.00 - .62
Nitrate (NO_3), ppm	.1 - 9.5	2.5 - 4.5
Boron (B), ppm	.01 - .15	.18 - .40
Dissolved solids, ppm	153 - 626	662 - 948
Hardness as $CaCO_3$, ppm	89 - 592	462 - 668
Dissolved oxygen (O_2), ppm	3.4 - 9.7	6.0 - 10
Carbon dioxide (CO_2), determined, ppm	2.0 - 23	44 - 88
Carbon dioxide (CO_2), calculated, ppm	1.5 - 22	11 - 79
Temperature (^{o}F)	50 - 66	55 - 58
Specific conductance, micromhos per cm at 25^{o} C	228 - 1,150	962 - 1,360
pH (field)	6.9 - 7.9	7.2 - 7.4
pH (laboratory)	7.1 - 8.3	6.9 - 7.7

1. A histological comparison was made between trout with and without gross (any urolithic calculi visible to the unaided eye) kidney urolithiasis to determine the extent of the deposition of calculogenic material in trout. These trout were collected from fish reared at the problem hatchery where the urolithic deposits occurred naturally.

2. Trout containing gross urolithiasis were transferred into a hatchery with different water chemistry to see if the deposits would regress, once formed.

3. Dry trout diets were altered in an attempt to induce urolithiasis in rainbow trout where no natural problem occurred. These investigations were started in the hope of being able to help determine causative factors under experimental conditions at the Logan Experimental Hatchery. A factorial experiment was eventually utilized to study possible influences in experimentally produced urolithiasis. Three main factors were investigated; two involved additions to the fish feed and the third involved different strains of fish. The diets were modified by adding two levels of sodium fluoride and also two levels of sodium bicarbonate. All possible combinations of these additives were mixed and pelleted in dry diets and fed to two different strains of fish for a 30 day period. After this length of time, a sample was checked for the number of fish containing gross urolithiasis.

4. The most calculogenic test diet, from the previous factorial experiment, was fed to albino rainbow trout in an attempt to determine the histological changes associated with experimentally produced urolithiasis. All fish (6) were sampled from one experimental unit

every four days and were checked for gross urolithiasis. Tissue samples were collected for subsequent histological examination.

OBJECTIVES

The objectives of this project were:

1. Investigation of naturally occurring urolithiasis found in rainbow trout (Salmo gairdneri) at the Midway hatchery.

2. Attempt to induce urolithiasis in rainbow trout reared in a water supply where no natural problem occurs.

3. Investigate the variables influencing experimentally produced urolithiasis by altering experimental diets and testing on different strains of fish.

4. Describe histology of naturally occurring and experimentally produced urolithiasis.

5. Suggest means which would minimize or eliminate the problem.

REVIEW OF LITERATURE

Mammals

Urolithiasis is a pathological condition that occurs in many different animals and is one of the oldest known afflictions of man. Archeologists have recovered urinary calculi from ancient Egyptians dating back to 4800 B.C. The most extensive studies have involved mammals, particularly humans and livestock. The seriousness of human urolithiasis is shown in the study by Bell (1946). He found a 1.12 percent incidence of urinary calculi in 25,000 human autopsies. This condition was listed as a cause of death in 0.38 percent of the patients. These results agree with other findings and most researchers feel that approximately 1.2 percent of humans have some type of stone. There are many problem areas termed "stone belts" where this percentage is greatly increased.

Though little work on urolithiasis has been done with fish, some similarities exist between fish and livestock. Urolithiasis is one of the most troublesome pathological problems facing the livestock industry. Large numbers of animals may be involved and extensive losses often result. In one flock of 2600 wether lambs, 10 percent were lost because of urinary calculi over a period of six weeks (Johnson, 1940). Livestock losses involve more sheep than cattle (Elan, Ham and Dyer, 1959), still urolithiasis is listed as the fifth most serious nutritional disease of cattle. The economic impact of urolithiasis in the livestock industry is also increasing. Newsom (1938) stated 30 years ago that urolithiasis was either becoming more prevalent or was more frequently diagnosed. Both seem to hold true. Modern feed lot conditions and feeding practices account for most of this increase. The persistent occurrence of calculi in ruminants, under feed lot conditions, suggests that certain feeds predispose animals to calculi formation (Cornelius, Moulton and McGowan, 1959). Concentrated diets that cause a large percentage of ruminants to develop calculi have been investigated and are now utilized in much of the present research involving experimental urolithiasis. Pelleting a concentrated experimental diet has been shown to cause a significant increase in the percent of sheep developing calculi (Robbins, 1938; Crookshank, 1965).

Many of the contributing factors involved in urolithiasis have been investigated by controlled production of urinary stones in animals (Vermeulin, 1951).

Increased occurrence and importance have resulted in many different types of urinary calculi being reported. Many circumstances and types of animals are involved and the research is often confusing and contradictory in regard to conditions necessary or resulting in calculi. The materials involved are composed of urinary constituents, but the factors responsible for stone formation are subject to many different opinions. Finlayson (1961), Figure 6, gives a good representation of the many variables influencing calculi formation. Calculi are composed mainly of an organic matrix material and a crystal material, either organic or inorganic. Much of the present controversy concerns the role of the matrix material. Vermeulin (1964) says, "The matrix is simply an adventitious inclusion in what is basically a mineralogic process and is neither necessary as cementing material or as a

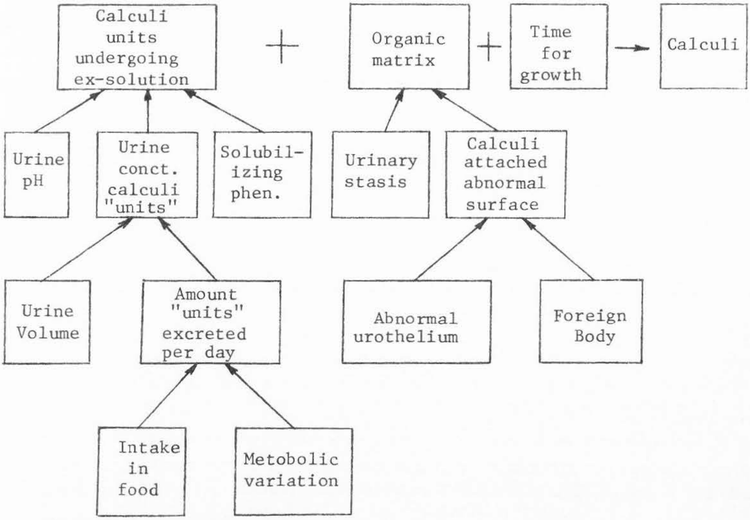

Figure 6-a. Variable factors, both known and speculative, influencing calculi formation in mammals (from Finlayson, 1961).

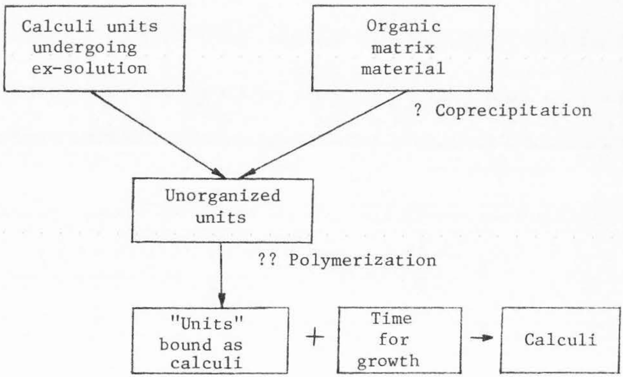

Figure 6-b. Interactions of matrix material involved in calculi formation in mammals (from Finlayson, 1961).

template." The opposite theory is that the "matrix is primary and stone formation is merely a consequence of its calcification" (Boyce and King, 1959).

Although the role of the matrix is contradictory, almost all urinary tract calculi contain an organic matrix throughout the stone. Small inorganic crystals form in urine, but the growth or formation of urinary calculi is in some way dependent on the protein matrix (Howard, 1954). All calculi found in warm blooded animals contain uromucoids, but the matrix proteins of different stones vary widely. The matrix may account for less than 2 percent to more than 6 percent of the dry weight of stones.

The crystalline composition of calculi often vary with the geographical occurrence. Inorganic type materials are found most frequently, but calculi composed of organic materials such as oxalate, uric acid, and xanthine are often reported. The inorganic mineral composition varies, both in types and amounts of minerals present. Calcium phosphate is the most common type calculus (Elliot, Sharp and Lewis, 1959). Apatite exceeds in bulk all other compounds found in American human urinary stones. A Russian study of human urinary calculi show the following minerals were present: Ca, Mg, Na, P present in excess; Fe, Ag, Al, relatively less, and Cu, Zn, Ba, Si, and Mo seldom or traces (Maksudov, Talipou and Parpiev, 1964). The composition of stones varies, and many other minerals are often found. Laminated stones may vary in composition from layer to layer.

Chemical analysis of the calculi is an important consideration in the investigation of any urolithic problem. It is often difficult to obtain sufficient material for a complete analysis. Prien and Frondel

(1947) recommends X-ray diffraction studies in addition to chemical analysis in order to obtain an insight into the matrix and crystal structure.

Weissman, Klein and Berkowitz (1959) used infrared spectroscopy to study the composition of various calculi. He obtained comparisons between different calculi and could produce rapid results using small amounts of material.

The chemical composition usually gives an indication of conditions involved in formation. Human calculi are often formed in acid urine and the deposits are usually well crystallized and have an organized matrix. Most of these cases are not associated with excessive mineral excretion (Lathem and King, 1963). In ruminants, however, stones usually form in alkaline urine and the deposits are frequently amorphous and the matrices poorly organized. Hard, discrete uroliths take long to form, while fine sand-like masses form rapidly (Hawkin, 1965). Neither type of urolithic formation is a simple precipitation.

Researchers now consider the formation of calculi as a symptom, not the disease (Baker and Sison, 1954). Present efforts are aimed at determining the conditions that result in the "diseased" condition resulting in urolithiasis. Many contributing factors have been studied and are thought to be involved through their possible effects of altering kidney function.

Vitamin A deficiency was implicated early in urolithic investigations (Van Leersum, 1928). Early evidence pointed to the deficiency causing epithelial cells to sluff and provide a nucleus for calculi formation (Runnells, Monlux and Monlux, 1965). Investigations have shown that Vitamin A deficiency alone (Lindley et al., 1953), or combined with mineral excess (Elan et al., 1959) is not sufficient to cause experimentally reproducible calculi in rats. However, the role of Vitamin A is still controversial.

Excessive Vitamin D has also been implicated. This may also be a contributing factor in certain circumstances, but is not a sole cause in itself.

The possibility of unknown harmful substances in the diets causing damage or altered function of the kidney was advanced by Hauschild (1963). These changes in the kidney could be a result of a decrease in the protective colloids in the urine. The extraordinary solubilizing ability of urine is in a large extent due to this protective colloid action (Butt, 1952). A change in these colloids may allow crystal growth and ultimately result in calculi. Many questions remain unanswered concerning the importance of colloids and minerals in urine. This area of urolithiasis seems to be very confusing and opinions vary regarding supersaturated versus unsupersaturated urine. The literature contains reports of calculi forming in both types. The urine concentration of calculi materials often does not exceed normal in many stone forms. Hunt (1963) measured calcium in the urine of humans, both normal and stone formers, and found no significant difference in calcium ratio. Lathem and King (1963) and Modin (1967) both found that urinary calculi may form rapidly in urine that is normal in concentration of urinary calcium. Johnson (1940) selected sheep known to be susceptible to formation of magnesium stones and fed diets high in magnesium and calcium. In spite of crystals forming in the urine, no calculi were produced, even with a three-fold increase in magnesium levels. In some experiments, increasing the level of dietary minerals may decrease the possibility of producing urinary calculi containing the increased minerals. The opposite is also true and urolithiasis often apparently results due to an increase of minerals in the urine. Vermeulin (1951), working with sheep, was able to induce or prevent urinary calculi containing certain ions by increasing or decreasing the concentration of those ions in the urine. Experimental diets causing the highest calcium load on the kidney of rats caused the highest incidence of calculi (Womack, 1967). Gross alterations in mineral metobolism have been associated with many of the experimental calculogenic diets (Packett and Hauschild, 1961).

Medication with sulfa drugs is also involved in certain cases of urolithiasis. Precipitated drug may provide a nucleus or template for calculi formation and sulfa crystals may cause damage to kidney tubules. It is important to accompany sulfa medication with sodium bicarbonate (Runnells, 1965). An alkaline urine is important because of increased solubility of sulfa drugs in alkaline solutions.

Packett (1956) reported a reduced number of sheep with urinary calculi when fed dietary chlortetracycline. This approach was investigated further by Robbins (1965) when he tested antibiotics added to feed. His results were opposed to the earlier work because he found chlortetracycline was not beneficial under similar circumstances.

Medication is probably associated with reduced incidence of urolithiasis through the control of infection. Bacterial contamination of urine increases the susceptibility to stone formation, even with a normal concentration of urinary constituents (Howard, 1954). Bacterial infection may accelerate deposit formation by altering urine pH.

Calcium type stones often result from urinary infections resulting in alkaline urine and a resulting decrease in solubility. Urine pH is an important factor and urine pH changes are utilized in cases of urolithiasis when attempting to dissolve as well as prevent calculi.

Seneca, Lattimer and Peer (1964) present the theory that absorption of gram negative endotoxins may initiate the formation of calculi. While this is only theory, the bacteria, Proteus, has been shown to be involved with deposits composed of uric acid through its urea splitting properties (Vermeulin and Goetz, 1954). They stress the importance of infection and recommend urine cultures of stone formers to determine if bacterial infection is involved. King and Boyce (1963) found a relationship between urinary tract infection and formation of experimental urolithiasis. However, urinary calculi have often been reported from aseptic urine, so infection alone will not cause deposits but can be an important contributing factor (Romanowski, 1965).

Viruses have been considered as possibly being involved in some cases of urolithiasis. However, no clear evidence has been presented to link viruses with urolithic problems.

Crowding and stress have been shown to increase susceptibility to calculi formation in lambs (Udal, 1959). Strain and sex of animals may likewise affect the composition and occurrence of calculi. Wexler (1963) found that different strains of rats have different susceptibility to stones. Vermeulin (1957) has shown in research using rats that different strains produce different types of stones under identical circumstances.

Despite extensive research on the problem of urolithiasis in mammals, many questions remain unanswered. Many related factors are involved, either as factors causing urolithiasis or they may be secondary to the presence of calculi.

Although the etiology of urinary calculi has been the object of a great deal of study, the subject is somewhat confused and contradictory evidence is common (Beeson, Pense and Holm, 1943). This statement seems to still be true and some investigators feel there may be as many factors involved in urolithiasis in mammals as there are types of calculi.

FISH

References of urolithiasis occurring in fish are seldom encountered in the literature. Tower (1902) found gallstones in weakfish (Cynoscion regalis). Hendrick (1961) found a large percentage of striped mullet (Mugil cephalus) containing "kidney stones." Reichinback-Klinke and Elkan (1966) reported the formation of calcium compounds in fish. Talbot (1967) reported on two urinary calculi found in striped marlin (Makaira audax). These were discrete, laminar stones composed of calcium and magnesium phosphate. Wunder (1967) described a condition of "kidney cysts" often occurring in rainbow trout (Salmo gairdneri). The condition he described appears similar to the problem of this study.

Visceral granuloma is a similar condition occurring in hatchery raised trout. The deposits are found throughout the viscera and kidneys and are often associated with diplobacteria. Snieszko (1961) reviewed the factors possibly contributing to the formation of visceral granuloma. The etiology of this disease also seems to be obscure with many possible interactions.

PREVIOUS EXPERIMENTAL RESULTS

This includes activities and investigations made by the personnel at the Experimental Hatchery relative to urolithiasis prior to this research project.

Post (1961) first encountered the problem of urolithiasis concerned with this study. He found calculi forming in the kidney tubules of rainbow trout (Salmo gairdneri) reared at the Midway Hatchery, Midway, Utah. Urolithiasis was no problem at this hatchery until changing to dry feeds as a sole sustaining diet.

Dr. George Post initiated several experiments (while employed by the Division of Wildlife Resources at the Experimental Hatchery) to see if the urolithic condition found at Midway could be prevented, arrested, or removed once formed.

Dr. Gar Workman (while employed at the same station) also conducted extensive research on the problem. Procedures were similar to those used in urolithic problems in warm-blooded animals. All experiments were conducted in the Midway water supply, where the problem occurs naturally.

Two modified diets were initially tested to see if these feeds would prevent urolithic problems in this water supply if fed from the "swim-up" stage. Rainbow trout (Salmo gairdneri) were used in all the experiments. Udal (1959) found force feeding of sodium chloride in pelleted rations prevented urolithiasis in sheep. Subsequently, sodium chloride was increased to 8 percent in a commercial trout diet to see if this would also work with fish. The second diet consisted of increasing 4 percent sodium sulfate in a commercial dry diet to possibly help the fish in its excretory processes. Each diet was tested with 5,000 fish in each replicate.

Neither diet prevented urolithic deposits, and a sample taken when the fish were six months old showed approximately 60 percent with visible kidney involvement. Additional diets were altered to see if other diet modifications would help dissolve or remove deposits that had developed during the first experiments.

The fish containing recently formed deposits were divided into four lots (two replicates in each lot) and given four treatments. All materials were added to a commercial dry diet and the four treatments were as follows:

1. Timmermann and Kallistrates (1966) reported that a few chelating agents had proven beneficial in removing calculi found in humans. A chelating salt Ethylenediamine Tetraacetate (EDTA) was fed to one lot of fish to see if this would prove beneficial.

2. Citirc acid was shown to be promising in urolithic problems in man, so this treatment was also selected.

3. The third lot received EDTA and citirc acid mixed to determine any combined beneficial effects. It was hoped that the citirc acid would dissolve the calculi and the EDTA would chelate calcium ions to prevent further deposition.

4. Ammonium chloride was fed to the fourth group to test the possibility of a diuretic removing calculi. It was hoped the increased urine flow would lessen the possibility of calcium salts saturating the urine, and thereby prove beneficial in controlling

calculi. Cornelius (1963) implicated a decrease in urine volume as being involved in urine calculosis.

All four treatments were continued for a period of six months. Fish were sampled at this time, and no significant differences in deposit regression occurred among the four treatments.

A new series of experiments were set up the following year. The citric acid and ammonium chloride diets were tested again to see if duplicate results would be obtained.

Mineral imbalance or overloading of minerals in the diet have been implied as contributing to urolithic problems under many various circumstances. Mineral substitution in the diet was attempted to determine any benefits. The wide calcium-magnesium ratio of the Midway water supply was a possible cause, so these minerals were altered in two diets.

One diet change consisted of removing the tricalcium phosphate and substituting sodium-biphosphate and magnesium chloride.

The other diet was similar except the tricalcium phosphate was removed, sodium-biphosphate substituted, and twice the level of magnesium chloride in the first diet was added.

A comparison between two different commercial dry diets was started at this time to determine possible differences due to type of diet. After feeding the experimentally modified diets for seven months, there were no significant differences in the number of fish developing urolithiasis. The fish averaged 48 fish per pound, and approximately 35 percent in each test group contained visible deposits. However, the comparison between the two commercial diets gave the first evidence that diet would influence the percent of fish with urinary calculi. One diet resulted in 5 percent of fish with visible deposits compared to 37 percent of the fish on the second diet. A third commercial diet was tested at the Midway station later the same year, and 36 percent of the fish were found to contain urolithic material in the tubules.

The negative results of the previous experimental diets, tested at the Midway Hatchery, indicated the urolithiasis problem was complex and likely due to more than one factor. These findings were similar to research involving warm-blooded animals that also suggested urolithic deposits are a result of multiple factors.

Previous sampling at the Midway Hatchery indicated an apparent seasonal increase in urolithiasis. It was speculated that water quality changes in the spring resulted in an increased occurrence. The composition of the water at the Midway Hatchery was investigated to determine any seasonal changes that might be a factor in urolithiasis. Water analysis data from the 11 state hatcheries was also compared to determine any constituents that were higher at Midway than the other hatcheries. Although the water supply at Midway is the hardest water used in any of the state hatcheries, the fluoride content was proportionally higher at Midway than at the remaining hatcheries. The Midway water contained 1.1 ppm fluoride when analyzed in July 1962, and it was possible that an additional spring increase could occur as a result of runoff from fertilizers containing fluoride.

The high fluoride levels in the water might be aggravated by additional fluoride contained in the dry feeds. Bone meal is fairly expensive and therefore defluorinated phosphate rock is used in dry rations. This phosphate may contain 0.04 percent fluoride and the combined effect of fluoride in both water and feed was thought to possibly be a contributing factor. Fluoride is also a cumulative poison and the long term exposure might result in eventual urolithiasis.

Pearman (1950) studied the possibility of enzyme inhibition causing urolithiasis in humans and it was thought fluoride might be involved because it is an enzymatic poison at certain levels. One of the enzyme systems fluoride can interfere with involves carbonic anhydrase. Inhibition of this enzyme results in an increased alkalinity of the urine. Urolithic deposits of calcium phosphate form in alkaline urine, and fluoride was possibly involved by this means. Because changes in urine pH may influence deposit formation, an alkalizer, sodium bicarbonate, was added to some of the experimental diets to determine the effects when fed to fish.

Preliminary work involved initial testing of several modified diets to determine if urolithiasis in trout could be induced in the Logan water supply. It was hoped it would be possible to study some of the contributing factors by attempting to induce calculi formation, utilizing experimentally modified diets. The water supply used at the Experimental Hatchery had not caused urolithic problems in the past when trout were raised and maintained on dry diets.

The results of these tests indicated the combined effect of sodium bicarbonate and sodium fluoride would cause gross urolithic deposits in rainbow trout (Salmo gairdneri) in a 20-day period. The factorial experiment used in this research was set up in an attempt to determine the significant influences in urolithiasis under the experimental conditions.

RESEARCH RESULTS

Midway Urolithiasis

Materials and methods

Rainbow trout (Salmo gairdneri) were collected from the Midway Hatchery and examined to determine the extent of mineral deposition in the tissues. Samples were collected from three rainbow trout containing extensive kidney deposits and compared with three normal rainbow trout from the same group of fish without kidney involvement. Tissue samples included heart, kidney, liver, spleen, dorsal muscle, fore gut, pyloric caeca, intestine, gill, and pseudobranch. Histological sections were cut, stained, and checked for similar deposits.

Bacterial cultures were incubated on Furunculosis Agar, Ordals, and enriched Ordals media (Difco) and all media was purchased in dehydrated form and reconstituted according to the manufacturers specifications.

Results and discussion

No deposits were observed in any tissues except the kidneys and pseudobranchia of the fish containing gross urolithiasis. Examination of fish at Midway showed gross deposits were found only in the kidney and pseudobranchia. These two locations are involved in excretory processes, and the material would be expected to be found there. The kidneys and pseudobranchia are also sites of carbonic anhydrase activity (Parry and Holliday, 1960) and this could also explain deposits forming in these tissues if an enzyme blockage were involved.

Although the kidney deposits were often extensive, there was apparently no mortality directly attributable to the urolithic condition. However, fish with urolithiasis might be susceptible to increased mortality because of the lowered resistance to secondary infections.

Fish kidneys contain more renal tissue than is normally necessary for body functions and this explains the ability for some fish to survive when grossly affected with urolithiasis. Eveleth et al. (1948) stated that unless urinary calculi, in lambs, interfere with passage of the urine, they do not seem to interfere with the health of the animal. A similar condition probably exists in fish, with the trout able to rely on their reserve kidney tissue to perform vital body functions.

A qualitative spectrographic analysis was initially performed on a sample of the trout kidney deposits. This test yielded the results shown in Table 2.

Table 2. Spectographic analysis of urolithic deposits removed from the kidneys of rainbow trout reared at the Midway Hatchery

Urolithic component	Quantitative analysis
Calcium	Major
Magnesium	Minor
Phosphorus	Minor
Silicon	Trace
Mn, Ba, Na, K, Cu, Ag, Fe, Al, B	Negative

Phosphorus is relatively insensitive in emission analysis, so it might have been present in major proportions and not detected. The trace of silicon was insignificant enough that the technician performing the tests thought it may have been a contaminant.

Additional analyses were attempted to obtain further information on the composition of the deposits. Three separate chemical analyses were started, and the results were similar and a composite is listed in Table 3. These deposits were collected from fish reared at the Midway Hatchery where the problem occurred naturally. The analysis on the organic portion of the deposits has not been completed.

Table 3. Quantitative analysis of urolithic deposits found in kidneys of rainbow trout reared at the Midway Hatchery

Urolithic component	Analysis Results
Loss on ignition (mostly organic matter)	33 %
Calcium	23.1 %
Phosphorus	14.3 %
Magnesium	2.4 %
Zinc	32 mg
Copper	20 mg
Ammonia	Absent
Carbonate	Absent
Iron	Absent
Cobalt	Absent

Deposits of this type are apatite in composition, and are invariably formed in alkaline urine. Apatite is reported as the most common component of urinary calculi in humans (Gershoff, 1964). Boyce and King (1959) recommended staining with Von Kossa stain to show apatite deposition. This stain was used on deposit material in histological sections and the results indicated the mineral was apatite.

Histological sections of kidneys containing deposits stained with hematoxylin-eosin (H & E) showed amorphous basophilic staining material located in the kidney tubules. The most extensive deposits were located in the collecting tubules, and in advanced stages, the tubules were greatly distended and ruptured. Extensive fibrosis also occurred in connection with the deposit material. The glomeruli remained intact, and the condition seemed to be confined to the tubules. The distal tubules are often filled with a basophilic material that stains as a muco-protein using the periodic acid Schiff stain.

Bacterial cultures were isolated from trout kidneys containing natural urolithic deposits and compared with cultures from normal fish from the same facilities. No consistent differences in bacterial colonies, either type or numbers, were noted between the two groups of fish. Many fish with advanced urolithiasis would show the kidneys free of bacteria.

Brown and Brenn bacterial stain was used to check for bacteria in histological sections taken from kidneys containing advanced urolithiasis. No bacterial concentrations were observed in any of the slides examined.

The only biochemical tests attempted on bacterial samples obtained from fish containing urolithic deposits were to check for bacteria of the genera <u>Proteus</u>. All samples tested negative as <u>Proteus</u>, and no additional tests were performed.

Bacterial contamination of the kidneys and urine apparently was not a necessary factor in formation of urolithiasis in trout. Bacterial contamination may be involved, and may possibly accelerate deposit formation by altering the urine pH, but similar to research with other animals it is not the sole determining factor.

At the time this portion of the study was completed, all fish at the Midway Hatchery were being fed the commercial diet which produced the least urolithiasis. This has reduced, but not entirely eliminated, the trouble.

Before the diet was changed at Midway, it appeared that as the feed companies refined and improved their dry diets even the most calculogenic feeds caused fewer gross problems. The response from personal correspondence with other state's hatchery men also indicates their problems have decreased since the first years of using dry feeds.

Conclusions

1. Naturally occurring urolithiasis at the Midway Hatchery involved kidney deposits consisting of apatite and an organic matrix.

2. Bacterial contamination of the kidneys and urine was not necessary for urolithic formation.

Regression Experiment

Materials and methods

On December 17, 1964, 1500 rainbow trout averaging 39.9 fish per pound were transferred from the Midway Hatchery to the Logan Experimental Hatchery. Preliminary sampling indicated approximately 40 percent of these fish contained gross urolithic kidney deposits. The fish were divided equally into two groups and each group was placed in a concrete fry pond. The fish remained in the fry runs for a period of four months after which time they were transferred into larger concrete raceways. In transferring into larger units the fish densities were maintained at levels that were comparable to the production densities found at the Midway Hatchery.

Each raceway of fish was supplied from the same $63^{o}F$ well water source at the Logan Experimental Hatchery (Table 4). Half of the fish were fed the same commercial diet (A) they had received at the Midway Hatchery prior to the transfer to the Logan Experimental Hatchery, and the other half were fed a different commercial diet (B). The fish were weighed and sample counted each month, and this data was used to adjust diet levels. The fish were fed twice daily.

Any fish that died during the experimental period was autopsied and checked for gross kidney deposits.

Results and discussion. None of the mortalities contained kidney deposits, so the decreased percentage of fish affected with urolithiasis was due to the fish eliminating the material.

Table 4. Analysis of water used for fish culture at the Logan Experimental Hatchery, based on samples collected on June 1, 1961

Chemical constituent or property	Analysis results (ppm unless specified otherwise)
Silica (SiO_2)	10
Iron (Fe), dissolved*	.00
Iron (Fe), total	.01
Manganese (Mn), dissolved*	
Manganese (Mn), total	.00
Calcium (Ca)	50
Magnesium (Mg)	21
Sodium (Na)	6.9
Potassium (K)	.8
Copper (Cu)	0.1
Bicarbonate (HCO_3)	245
Carbonate (CO_3)	0
Sulfate (SO_4)	13
Chloride (Cl)	7.0
Fluoride (F)	.1
Ammonia (NH_4)	.08
Nitrate (NO_3)	1.2
Nitrite (NO_2)	.04
Boron (B)	.03
Dissolved solids	
Sum	235
Residue on evaporation at 180° C	223
Hardness as $CaCO_3$	210
Non-carbonate	9
Alkalinity as $CaCO_3$	201
Specific conductance (micromhos at 25° C)	405
pH	7.7
Color	5
CO_2 (calculated)	7.8
Total inorganic PO_4	.04
Temperature (°F)	63
Dissolved oxygen (O_2), ppm	7.4

* In solution at time of analysis.

The results of the experiment, after six months, show a decrease in the percent of fish with gross urolithiasis from the original incidence (Table 5).

Table 5. Comparison of the reduced incidence of urolithiasis, in rainbow trout, resulting from a change in water quality only or a change in both water quality and diet

Original percent of fish with urolithiasis	40%	40%
Diet	Same feed "A"	New feed "B"
Fish/pound	3.56	2.73
Percent of fish with urolithiasis	23%	13%

The experiment was continued for an additional six month period, and the percent of incidence followed a similar decrease with both diets.

A change in water chemistry or quality (from Midway Hatchery water into Experimental Hatchery water) was sufficient to cause gradual deposit elimination; however, with a change in diet to a less calculogenic feed, the kidney deposits regressed faster.

The fish in this test were fed to achieve good growth, and they had increased about 50 percent in size in the six month period. Samples collected after the second six month period showed most of the urolithiasis gone with fibrous tissue replacing the deposits.

Conclusions

1. Kidney deposits gradually regress if affected fish are placed in water supplies containing less dissolved solids.

2. The deposit material regressed faster when the fish were fed a less calculogenic diet in addition to the changed water chemistry.

Experimental Urolithiasis

Materials and methods

Experimental facilities. All experiments attempting to induce urolithiasis were conducted in the water supply of the Experimental Hatchery at Logan, Utah. The water flows from artesian wells, has a constant temperature, and is pathogen free. The water originates at two different levels resulting in two water temperatures. The water used outside was $63^{o}F$, while the supply inside the hatchery building was $57^{o}F$. The water quality and chemistry are similar from both levels, with the only major difference being temperature.

Experimental equipment. The experiment units consisted of 50 gallon barrels set in a series within outside concrete raceways. Prior to use, the barrels were sand-blasted and the inside painted with two coats of epoxy paint. A double standpipe arrangement was used so that water could be drawn from off the bottom of the barrels to aid in keeping them clean. A slight circular water motion was maintained to aid in accumulating wastes in the center and facilitate removal out of the stand-pipe. Fresh $63^{o}F$ water entered at the surface, with a one-foot fall from the pipe. Each unit was adjusted to contain five cubic feet of water and fish densities were one pound of fish per cubic foot of water. All barrels were operated at four exchanges of water per hour, and this continual exchange maintained good water quality throughout the test. All units worked satisfactorily and were self-cleaning and trouble-free during the experiments.

The barrels were covered with one-half inch mesh hardware cloth. This prevented fish from escaping and still allowed access for feeding.

Experimental fish. All fish used in this experiment were purchased as eyed eggs, shipped to the Experimental Hatchery and hatched in 57°F water. When the fish reached approximately 1000 fish per pound, they were transferred outside into concrete raceways, with a water temperature of 63°F. All experimental fish were fed commercial diets and were healthy and apparently disease free. No mortality problems were encountered during the experiments.

Two strains of fish were utilized in the experiments, and fish used in the tests were randomly selected from larger lots of fish. White's strain of rainbow trout were purchased from White's Trout Farm in Paradise, Utah, and were from the Hull-Erickson strain. The fish were 8.9 fish per pound when selected for the experiments. The Kamloop strain of rainbow trout were purchased and shipped from Soap Lake, Washington. These fish were 11.6 fish per pound, when used for the experiments.

Experimental diets. All feed additives were added to pulverized commercial dry trout feeds and mixed thoroughly before pelleting. The fish size determined both the size of feed pulverized and pellet sizes after mixing. All experimental feeds were pelleted using water as a binding agent and were air dried. The control feed consisted of a commercial dry diet, pulverized, pelleted, and air dried without additives.

Two levels of fluoride and two levels of bicarbonate were used in the experimental diets. The low fluoride level was .833 grams per

per pound of feed and the high level was 1.666 grams per pound. The bicarbonate levels added to the diets were 50 grams per pound of feed on the low level and double that amount (100 grams per pound of feed) in the high level. All possible combinations of these treatments were added to test diets. This involved nine separate experimental diets including the control feed.

Experimental design. A randomized block 3 x 3 x 2 factorial experiment was utilized to determine the significant factors contributing to experimental urolithic formation. Each modified diet was tested on the two strains of fish, and this resulted in a total of 18 separate treatment combinations. Two replications were used for each treatment and 12 fish were sampled from each replicate.

Experimental procedure. Test fish were placed into the experimental units seven days before feeding the experimental diets. During this adjustment period, a regular commercial pelleted diet was fed twice daily. The feed levels were 100 percent standard chart level, and this amount was determined by fish size and water temperature. All feed levels were corrected for any mortalities that occurred in any of the units. Mortalities were checked for occurrence of gross urolithiasis only.

When the experiments were terminated after 30 days, fish to be autopsied were anesthetized in MS-222 (tricane methane sulfonate). A routine autopsy was performed, and this consisted of visually checking gills, pseudobranchia, liver, fat, spleen, and kidneys. All tissues sampled for histological examination were dissected out and immediately placed in 10 percent neutral formalin. At least ten times the volume of fixative to tissue was used, and samples remained in formalin for a minimum of 24 hours before sectioning. The paraffin method of embedding was used, and all sections were cut at seven microns. Standard hematoxylin-eosin, periodic acid-Schiff (PAS), Von Kossa, and Brown and Brenn stains were used and cover slips mounted with permount.

Results and discussion

Bicarbonate - $NaHCO_3$. The experimental fish showed a wide range of response to the various treatments (Table 6). When an analysis of variance was applied to the experimental data, only the bicarbonate treatment tested significant (Table 7). The wide range of response resulted in a large error term, and this may mask some of the potentially significant effects.

The bicarbonate treatment was significant at the 5 percent level (Table 7), and was even significant at the 1 percent level. This significant effect of the addition of the alkalizer sodium bicarbonate to the diet apparently resulted in an increase in urine pH sufficient to cause urolithiasis in many of the fish (Figures 7 and 8). No measurements were made to determine the actual changes in urine pH due to the modified diets. The amount of sodium bicarbonate added to the diet was excessive and resulted in gross urolithiasis after only two weeks. If modified diets can rapidly cause urolithiasis in water supplies normally free of the problem, then the possibility exists of gradual urolithiasis formation in problem "hard water" supplies because of dietary induced altering of urine pH. Even a slight basic urine pH

Table 6. Experimental design and results for experiment involving inducement of urolithiasis. Each replicate total represents the number of fish, out of a sample size of 12, that developed gross urolithiasis after being fed the experimental diets for a 30 day period

Experimental diet additives per pound feed	White's Strain Rep. #1	Rep. #2	Soap Lake Strain Rep. #1	Rep. #2
Control (no additives)	0	0	2	0
50 grams $NaHCO_3$	0	0	0	0
100 grams $NaHCO_3$	1	0	1	1
.83 grams NaF	1	1	5	2
1.66 grams NaF	3	7	7	6
50 grams $NaHCO_3$, .83 grams NaF	6	1	5	3
50 grams $NaHCO_3$, 1.66 grams NaF	6	2	2	6
100 grams $NaHCO_3$, .83 grams NaF	4	5	2	5
100 grams $NaHCO_3$, 1.66 grams NaF	4	1	5	5

Table 7. Analysis of variance of number of two different strains of trout developing gross urolithiasis when fed diets containing added sodium bicarbonate and/or sodium fluoride for a 30 day period

Source of variation	Degrees of freedom	Mean square	F test value
Replications	1		
Treatments			
Sodium bicarbonate	2	49.0	15.65*
Sodium fluoride	2	3.0	.96
Strain of fish	1	6.25	2.00
$NaHCO_3$ x NaF	4	5.0	1.60
$NaHCO_3$ x strain	2	1.0	.31
NaF x strain	2	1.0	.31
$NaHCO_3$ x NaF x strain	4	1.75	.56
Experimental Error	17	3.13	

* Significant at 5 percent level.

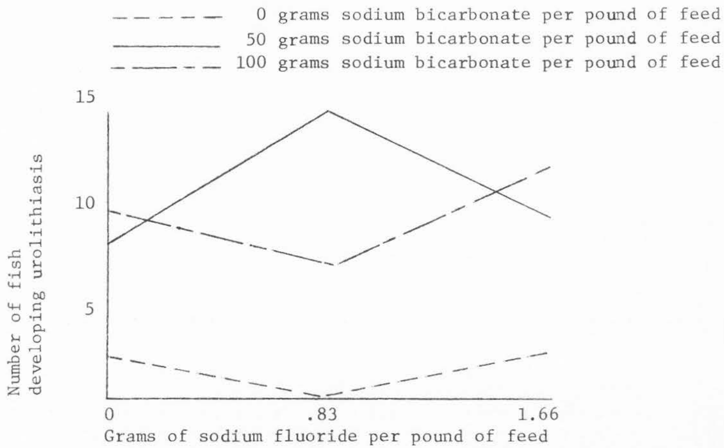

Figure 7. Number of Soap Lake strain of fish developing gross urolithiasis when fed sodium bicarbonate and/or sodium fluoride in the feed. Twenty-four fish sampled at each level of sodium fluoride.

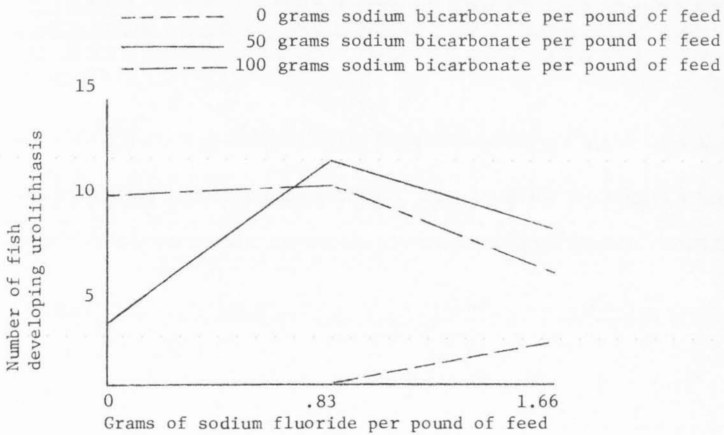

Figure 8. Number of White's strain of fish developing gross urolithiasis when fed sodium bicarbonate and/or sodium fluoride in the feed. Twenty-four fish sampled at each level of sodium fluoride.

change above normal levels may eventually contribute to development of kidney urolithiasis.

There are many ways the diet could possibly affect the ultimate pH of the urine, but the only two investigated were enzymatic inhibition and excessive alkalizers. The level or availability of proteins in dry diets is also involved in urine pH and this is a possible contributing cause of urolithiasis that was not investigated.

The significant response of the addition of an alkalizer to the diet indicates the importance of a feed that maintains the urine pH within the normal range. The results of this experiment also imply that a possible means of preventing apatite deposit formation in the kidneys would be to lower the pH until the urine is acidified. It may be feasible to add an effective acidifier to pelleted dry diets in an attempt to prevent urolithiasis at problem hatcheries.

Fluoride. Sodium fluoride added separately to the diet was not a significant treatment under the conditions of this experiment (Table 7). The addition of the high level of fluoride to the feed caused only a slight increase of urolithiasis.

The bicarbonate x fluoride response was not significant at any level below the 25 percent level. In spite of this lack of significance the highest response, in both strains of fish, to any of the treatments, occurred in the combined low levels of bicarbonate x fluoride diet (Figures 7 and 8). This result was also observed during preliminary testing of experimental feeds. This trend of a higher response to a combination of diet additives was consistent and reproducible throughout the experiment. Although this study did not show a statistically significant difference, the consistent high response when fluoride was added indicates a possible involvement in urolithiasis (Figures 9 and 10). At least a combination of effects appears to increase the percent of fish developing urolithiasis. This observation is also in general agreement with the prevalent opinion that urolithiasis formation results due to a combination of factors, rather than one particular cause.

Strain of fish. The strain of fish (Table 7) as an influencing factor in development of urolithiasis was significant only at the 25 percent level. This lack of significance also appears due to the variation among fish and the resulting large error term. However, trends in the response of the two strains were again consistent and reproducible with the Soap Lake strain even showing the presence of urolithiasis in the control fish (Table 6). This occurrence, and the larger numbers involved, suggest that the Soap Lake strain is more susceptible. This strain was consistently higher in number of fish with gross urolithiasis (Figures 11 and 12). The response to the treatments also varied more in the Soap Lake strain (Figures 7 and 8). Figure 7 indicates an apparent interaction in the Soap Lake fish between the low and high levels of bicarbonate, but there was no significance. Larger sample sizes would help establish any significant differences between strains.

There was a difference in the average size of fish in each strain, and this could also contribute to the higher response in the Soap Lake fish. The White's strain were larger (9 fish per pound) than the Soap Lake fish (11 fish per pound). During the experimental work, it consistently appeared that size of fish had an influence on the numbers that developed urolithiasis. The smaller fish seemed more susceptible

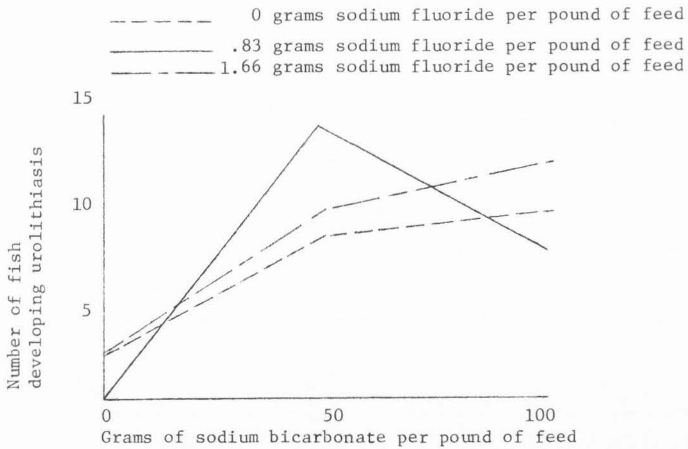

Figure 9. Number of Soap Lake strain of fish developing gross urolithiasis when fed sodium fluoride and/or sodium bicarbonate in the feed. Twenty-four fish sampled at each level of sodium bicarbonate.

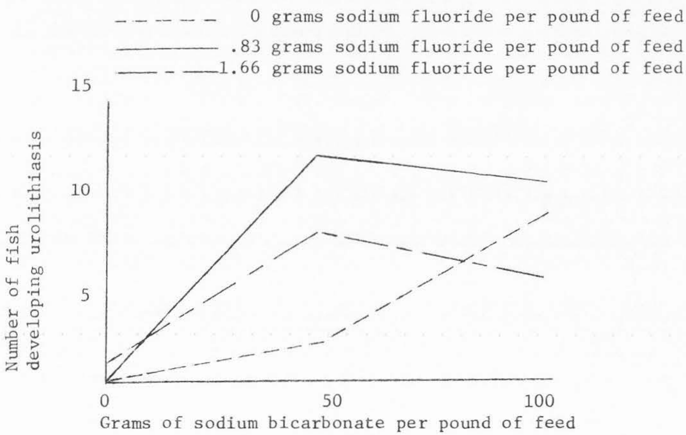

Figure 10. Number of White's strain of fish developing gross urolithiasis when fed sodium fluoride and/or sodium bicarbonate in the feed. Twenty-four fish sampled at each level of sodium bicarbonate.

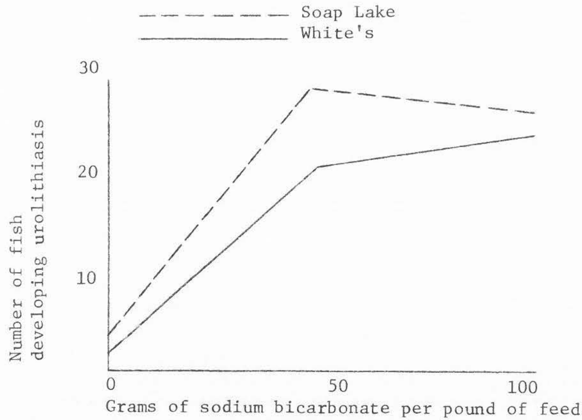

Figure 11. Number of fish of each strain developing gross urolithiasis when fed sodium bicarbonate in diet. The response is independent of fluoride levels in the feed. Forty-eight fish sampled at each level of sodium bicarbonate.

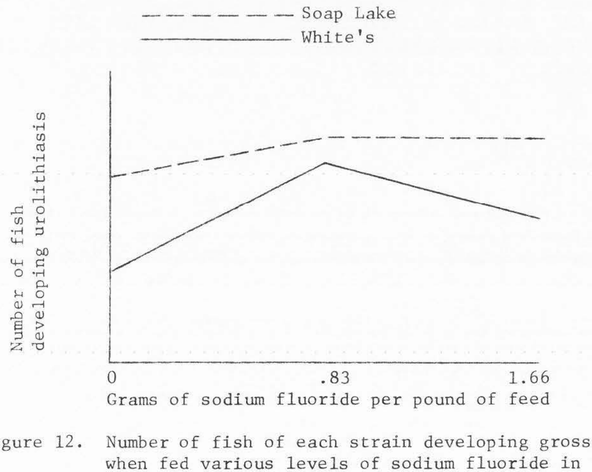

Figure 12. Number of fish of each strain developing gross urolithiasis when fed various levels of sodium fluoride in the feed. The response is independent of the sodium bicarbonate levels in the feed. Forty-eight fish sampled at each level of sodium fluoride.

to formation of kidney deposits than larger fish. Although this was only an observation, it appeared that during the trial experiments and subsequent late experimental work, that as the same diets were fed to larger fish the urolithic incidence decreased. It was apparent when each experimental group of fish was autopsied that the incidence of urolithiasis was higher in the smaller size of fish. If the smaller size fish are more susceptible, this would be similar the the problem in rats, where Coburn and Packett (1962) found young rats were more susceptible to stone formation than mature rats. These observations suggest that it would be advisable to transfer the largest fish possible into a problem water supply. The more size they could obtain prior to being placed into problem water, the less their chances would be of developing urolithiasis.

It should be noted that not all fish are affected by the disease. This deduction can be arrived at by observing that:

1. In the affected hatchery, under identical conditions, the percent of fish developing urolithiasis varies.

2. Under observations conducted in this series of experiments again the same differences occur and not all fish were affected.

The factorial experiment was not without errors and shortcomings, and the following are possible ways to reduce the variation in the results:

1. Select more uniform size of fish. Set a narrow length-weight range. Steps need to be taken to make certain the two different strains of fish were the same size and fish within strains were similar. A wide variation in size of fish is possible if only using the average figure of fish per pound.

2. Set up larger experimental lots of fish and select larger sample sizes.

3. Another approach would be to mark and keep track of individual fish in an experiment. By keeping a record of each fish, its separate length and weight before and after the testing, it would help correlate gains in weight with formation of urolithiasis. This method would indicate if fish had refused diets, or possibly not obtained the quantity of feed as fish developing urolithiasis.

Conclusions

1. Experimentally, urolithiasis in trout can be produced utilizing calculogenic diets, and may be useful to investigate some of the variables involved in urolithiasis.

2. Addition of an alkalizer ($NaHCO_3$) to fish diets was statistically significant at the 5 percent level as an influence in causing urolithiasis in trout.

3. Trends were consistent and apparent, although not statistically significant under the experimental conditions that strains of fish vary in susceptibility to urolithiasis.

Albino Histological Experiment

Materials and methods

Experimental facilities. Six one-gallon modified plastic jars were set up as experimental units for the smaller fish. The jars were placed inside the Experimental Hatchery building in fiberglass hatchery troughs. The overflow pipe was arranged so that each unit contained 0.1 cubic feet of water. Water at 57^{o}F entered at the bottom of the jar and overflowed the outlet at the top. Each unit contained six fish and was maintained at four exchanges of water per hour.

Experimental fish. The albino rainbow trout used in this experiment were purchased as eyed eggs from the Ennis National Fish Hatchery, Ennis, Montana. The eggs were hatched, and the fish reared inside the hatchery building in 57^{o}F water. The fish averaged 170 fish per pound when the experiments were started.

Experimental diets. The most calculogenic experimental diet from the previous factorial experiment was used exclusively in this experiment. This feed contained the low levels of both fluoride and bicarbonate.

Experimental design. Six fish were placed in each of six jars. Each jar of fish was fed a regular diet prior to being fed the experimental diet. One jar of fish was designated as a control unit wherein the six fish were to be checked for urolithic formations after the seven days of regular diet and prior to receiving the experimental diet. Fish in each of the five remaining jars were fed the experimental diet. Thereafter, one jar of fish was selected every four days, and the fish checked for urolithiasis.

Experimental procedure. The fish, when on the experimental diet, were fed four times a day. The feed levels were also 100 percent chart level.

All fish (6) in one unit were sacrificed every four days and were visually checked for urolithiasis. Kidney tissue samples were collected and quickly placed in 10 percent neutral formalin. After 24 hours, these sections were embedded, sectioned, and stained using standard histolotical techniques and stains.

Results and discussion

This experiment was an attempt to determine and follow the histological changes associated with urolithic formation. Albino rainbow trout were utilized because the absence of pigment in the kidney tissue would make changes more noticeable. Six fish were placed in each jar, and although no mortalities occurred during the experiment, four fish escaped and this resulted in a modified sample size (Table 8).

The first histological deposits formed after only eight days and gross urolithiasis was observed after only 12 days. Urolithiasis has also been rapidly produced in other test animals using calculogenic diets. Cornelius et al. (1959) has shown that crystals of calcium phosphate form in the urine of lambs after only 24 hours. When the conditions resulting in urolithiasis are present, the deposits evidently can form rapidly.

Table 8. Incidence of gross and histological urolithiasis in albino rainbow trout fed experimental calculogenic diet for various lengths of time

Sample Date	Elapsed Days	Number fish Sampled	Gross Deposits	Histological Deposits
4/ 8	0	6	0	0
4/12	4	4	0	0
4/16	8	5	0	2
4/20	12	6	2	2
4/24	16	5	4	5
4/28	20	6	4	5

The deposit formation evidently occurs by the following means. The excretory fluid flows through the kidney tubules and it becomes more concentrated and material precipitates out in the lumen of the tubules. The material continues to build up as more deposit material precipitates. The kidney tubules react by dilation and as more material forms, the dilated tubule finally ruptures. Fibrocytes (scar tissue) also build up in connection with the mineral deposits and form the typical urolithic deposit in the kidneys.

The natural and experimentally produced urolithiasis appear similar, although no chemical analysis was attempted on the experimental deposits. Both kidney deposits stain basic with standard H & E stains, and both have the same gross appearance. They each have a similar amorphous structure under low magnification. The deposits are formed in similar sites in the kidneys, with both distal and collecting tubule involvement.

Bacterial contamination did not vary between fish containing experimental urolithiasis and fish not developing deposits. Cultures were sampled on furuncolosis agar using ten fish containing visible urolithic deposits. These fish showed no bacterial growth from the kidneys.

Conclusions

1. Albino rainbow trout can be useful for histological investigations due to the lack of pigment in tissues.

2. The formation of experimentally produced urolithiasis in albine rainbow trout was confined to the tubules of the kidney and pathological changes occurred only in this portion of the kidney.

GENERAL DISCUSSION

The variable results of these experiments and the conflicting reports in the literature both seem to implicate more than one cause in urolithiasis. The calculogenic nature of concentrated dry feeds was also propably a result of many factors. The commercial feed companies are continually refining and improving dry feeds, and this has evidently reduced the urolithiasis troubles. A survey to determine the extent of similar problems, occurring in hatchery reared trout, was conducted by writing state and federal installations. The response indicated that many other hatcheries have experienced similar conditions in certain lots of trout. Urolithiasis occurred or was reported by approximately 40 percent of the agencies contacted (personal correspondence). Most of these answers indicated their urolithiasis problems apparently decreased since the early days of using dry diets.

The problem at the Midway Hatchery has improved somewhat since the first occurrence. There are many possibilities to explain this reduction, but any or all of the following factors may have been involved. The number of fish reared at the hatchery has been reduced, and this has helped eliminate stress; the fish are of a better quality and are not as susceptible to the former disease problems; the reduced bacterial infection problem may account for the reduced urolithiasis; with less bacterial problems, the feeding of medicated feed has been eliminated, and the previous sulfa treatments may have been involved in urolithiasis because of changes in urine pH.

The water quality has improved because of the reduced fish numbers, and the hatchery superintendent has also increased the water exchange by lowering the pond water levels. This increased exchange has reduced the ammonia build-up in the ponds and lessened any possible ammonia involvement.

The improved fish quality has also been complemented by the improvement of dry feeds. By continuing to improve dry fish diets, the present difficulties at certain hatcheries will eventually be eliminated, and the improvement and perfection of dry feeds will continue to increase the potential of fish culture.

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